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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Nancy T. Chang

Serial No.: 659,339

Filed: October 10, 1984

Title: CLONING AND EXPRESSION OF HTLV-III DNA

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 5-14-86

Hamilton, Brook, Smith & Reynolds

Ellen Ritrovato
Signature

5-14-86
Date

DECLARATION OF NANCY T. CHANG

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Nancy T. Chang, of 7405 Brompton St., Houston, Texas 77025, declare:

1. I am an inventor of the subject matter described and claimed in the above-identified application. When the invention was made, I was

Affidavit Exhibit 2
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Associate Research Director in Molecular Biology at Centocor, Incorporated, Malvern, Pennsylvania (Centocor), assignee of the subject application. Currently, I am an Associate Professor of Medicine at Baylor College, Houston, Texas.

2. At the time the application was filed, Dr. Robert C. Gallo and Dr. Flossie Wong-Staal were not designated co-inventors when, in fact, they were co-inventors and should be so designated.

3. The above-identified application discloses and claims methods for cloning and expressing subgenomic fragments of HTLV-III cDNA; HTLV-III cDNA fragments and immunoreactive HTLV-III polypeptides encoded thereby; and methods of detecting antibody against HTLV-III employing the polypeptides.

4. The experimental work described in the application began at Centocor upon receipt of genomic HTLV-III DNA from the laboratories of Dr. Gallo and Dr. Wong-Staal. Dr. Gallo and Dr. Wong-Staal supplied a recombinant phage (designated λBH 10) consisting of the genomic HTLV-III cDNA recombined with a phage vector. The HTLV-III cDNA insert was excised from λBH 10 and fragmented and the subgenomic fragments were cloned and expressed in host cell systems as described in the application. All of the experimental work described in the application was done at Centocor, either by me or by laboratory assistants working under my direction and supervision. However, at various times before the experimental work and during its progress, Dr. Gallo, Dr. Wong-Staal and I discussed the strategy

for the cloning and expressing of the viral cDNA. The experimental work proceeded along the lines we discussed; thus Dr. Gallo and Dr. Wong-Staal contributed signficantly to the cloning and expression of the HTLV-III cDNA.

5. On August 22, I prepared a document which described the experimental work accomplished up to that time. The document was sent to Centocor's patent law firm, Hamilton, Brook, Smith & Reynolds (HBS&R), as an "invention disclosure" (Exhibit A). Because all of the work described in the "invention disclosure" document was done at Centocor and because of my incomplete understanding of the law of inventorship, I did not designate Dr. Gallo or Dr. Wong-Staal as "inventor" on this document.

6. Subsequently, Centocor decided to have a patent application prepared and filed by HBS&R. Because of the imminent publication of an article disclosing work relating to the invention, there was great urgency to file the application. On October 8, 1984, I met with Centocor's patent attorneys to supplement information contained in the "invention disclosure" document (Exhibit A) for completing of a patent application. At this meeting, all of my time was devoted to explanation and discussion of the highly technical and complex subject matter necessary to prepare the application. The subject Application, Ser. No. 659,339 was filed on October 10, 1984.

7. On January 23, 1985, a continuation-in-part application was filed to cover additional experi-

mental work which had been done since the earlier application was filed. The inventorship error was repeated.

8. The possibility of an error in inventorship was first raised by Dr. Gallo in a letter to me dated July 25, 1985 (Exhibit B). Shortly thereafter, Centocor management initiated an investigation into the facts surrounding the invention and authorized HBS&R to do the same (Exhibit C). After a preliminary investigation, Centocor management made a tentative response to Dr. Gallo on September 16, 1985 (Exhibit D). However, pursuant to Centocor's stated desire to have the patent legally drawn a thorough investigation was made. During this investigation, I informed HBS&R of the full extent of Dr. Gallo's and Dr. Wong-Staal's collaboration with me regarding conceptual aspects of the claimed subject matter. After consideration, HBS&R concluded that Dr. Gallo and Dr. Wong-Staal should be designated as co-inventors because of their conceptual contributions.

9. My earlier failure to indicate the contributions of Robert C. Gallo and of Flossie Wong-Staal was unintentional.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nancy T. Chang

Nandy T. Chang

Feb. 23, 1986

Date

EXHIBIT A

THE CHARACTERIZATION AND PRODUCTION OF HTLV-III GENES AND PROTEINS BY GENETIC ENGINEERING METHODS

Nancy T. Chang

Centocor, Inc.

244 Great Valley Parkway

Malvern, PA 19355

August 22, 1984

Nancy T. Chang
Aug. 27, 1984

8/27/1

Witnessed by

Aug 27, 1984

Documentary Exhibit 5
CHANG ET AL.
Interference No. 103,659

Diagnostic and Vaccine Developments for AIDS

Human T Cell Leukemia Virus type III (HTLV-III), also named Lymphoadenopathy Virus (LAV), was isolated from the peripheral blood or lymphoid tissues of patients with Acquired Immune Deficiency Syndrome (AIDS). Recent studies of R. Gallo's group and of L. Montagnier's group indicated that the sera from over 80% of AIDS and pre-AIDS patients contain antibodies specific for the viral envelope and core proteins of HTLV-III. This and other evidence strongly suggests that HTLV-III is the cause of infectious AIDS, giving hopes that the diagnosis, preventive vaccine, and even therapy for AIDS can soon be developed.

Because AIDS can be transmitted through blood transfusions, an assay that detects HTLV-III infection is important not only for diagnosing patients but also for screening blood that might be contaminated with the virus. NIH and several commercial firms, including Centocor, are now developing immunoassay kits employing inactivated, disrupted HTLV-III as the solid-phase immunoadsorbent for the detection of antibodies against HTLV-III antigens in serum or blood.

Genetic Engineering Approach

Another approach for the detection of and the vaccination against HTLV-III infection is the employment of genetic engineering methods. In this approach, the proviral genes integrated into host cell DNA are molecularly cloned. The nucleotide sequence of the molecular cloned provirus is determined. The viral nucleotide sequence information will be directed to design and engineer HTLV-III-specific peptides and DNA probes using recombinant DNA technology or synthetic peptide chemical synthesis methods. These products are then explored for use in the diagnosis of HTLV-III infections by measuring specific antibody to the viral peptides or HTLV-III-specific RNA or DNA. The peptides, especially the gag and env related peptides may also be used as vaccines for the prevention of AIDS.

More specifically, the env and gag genes, which encode the envelope and core proteins of HTLV-III, respectively, are subcloned into various bacterial or mammalian expression vectors. These expression vectors contain all the necessary controlling elements for the production of the fused HTLV-III env gene in recombinant plasmids bearing host cells. Expression of the HTLV-III related peptide in the foreign host cells can be detected by binding of HTLV-III specific antibody in the AIDS patient serum or hyperimmune serum raised against purified virus. Although the env and gag products are of primary interest for diagnostic and vaccine purposes, the other two genes encoded by HTLV-III, pol and Px are important for understanding the biology of this retrovirus. These genes will be studied as well.

The genetic engineering approach offers a few advantages over the conventional one, which involves growing HTLV-III in cell cultures. For example, in the manufacturing process, because viral antigens are not infectious, working personnel are not exposed to the hazardous virus and the facility requirement will be less stringent than that for virus production. Also, the envelope and core proteins are the dominant immunoreactive viral antigens, immunoadsorbents with the purified viral proteins may offer more antibody-adsorbing capacity and higher sensitivity than those with whole virus. Immunoassays employing envelope and core proteins separately can detect antibodies against envelope and against core proteins. The antibody profile (concentrations and proportions) may reveal certain natures of the disease yet to be discovered. Furthermore, a protein vaccine using purified viral proteins (env or core gene product) will not have the risk of viral infectivity.

Centocor's First Footstep in HTLV-III Molecular Biology Work

As soon as we obtained the information in early May, 1984, that HTLV-III was isolated from AIDS patients and shown convincingly to be the cause of AIDS and that antibodies against HTLV-III antigens were found in over 85% of AIDS and Pre-AIDS patients. I decided to use the genetic engineering approach to develop diagnostic assays for AIDS. On May 10, 1984, Tse Wen Chang, Michael Wall and myself went to Biotech Corporation, Rockville, Maryland, to meet Dr. Robert Ting (Chairman of Biotech) to discuss the collaboration between Centocor and Biotech about coating polystyrene beads with inactivated disrupted HTLV-III. In that meeting, I expressed my interest to clone and

express HTLV-III genes and to use the expressed proteins for diagnostic and vaccine products. Dr. Ting was impressed with our expertise in Molecular Biology and introduced me to Dr. Flossie Wong-Staal, a key associate of Dr. Robert Gallo, with whom he had been collaborating on certain aspects of HTLV-III work. Our collaboration with the NCI group started on that day. We returned to Centocor with E. coli clones encoding segments of HTLV-II DNA. At that time, HTLV-III DNA had not been cloned.

The collaboration between Centocor and the NCI group went on very nicely. On July 5, we visited Dr. Wong-Stahl reporting our progress on HTLV-II and proposing our strategy on HTLV-III. We obtained λ clones harboring a segment of HTLV-III DNA on July 20, 1984. Our work on HTLV-III started on that day.

Centocor's Progress Update

We now have E. coli plasmid clones containing various portions or entire genome of HTLV-III. We have sequenced a segment (about 3500 base pairs long) of HTLV-III genome encoding most of the env gene. We have also cloned HTLV-III DNA in several expression host-vector systems and obtained several clones that can be induced to synthesize polypeptides encoded by the inserted HTLV-III DNA. Efforts are being made to test the reactivity of these polypeptides with antibodies from AIDS patients. When we identify clones that produce polypeptides demonstrating good reactivity with the antibodies, we will produce the polypeptide in large quantities and use it in immunoassay development. We also plan to clone and express the gag gene in a few weeks.

Plans are also being made to transfect mammalian cells with the E. coli cloned env and gag DNA's.

The Application of HTLV-III Related Peptides or Proteins

The viral envelope and core related peptides produced by the env and gag clones, either separately or combined, can be coated or conjugated noncovalently or covalently onto solid phase, such as PVC plate or polystyrene beads to be used as immunoadsorbent for antibodies against them. These solid phase immunoadsorbents are the key components in the immunochemical assays for HTLV-III-specific antibodies, using tracers such as goat anti-human immunoglobulin or protein A that are conjugated with radioactive isotopes such as ^{125}I, or enzymes such as peroxidase or alkaline phosphatase.

The proteins can also be used to prepare vaccine against HTLV-III, which should be useful for high-risk populations, such as homosexual males and recipients of frequent blood transfusions. The genetic engineered envelope and core proteins can also be used as an immunogen to prepare monoclonal or polyclonal antibodies. These antibodies can be employed in immunochemical assays for the detection of viral antigens in serum, blood, lymphocytes, or other tissues of AIDS or pre-AIDS patients.

The nucleotide sequences of HTLV-III env and gag genes yield information about the amino acid residue sequences of the envelope and core proteins.

Artificially synthesized segments of polypeptides according to the sequences may offer potential in diagnostic assays and in vaccines.

The cloned HTLV-III and its sequence can also be used to prepare DNA probes for the detection of HTLV-III RNA, proviral DNA, or encoded mRNA in the lymphocytes, or other tissues of patients.

EXHIBIT B

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20205
Building : 37
Room : 6A09
(301) 496-6007

July 25, 1985

Dr. Nancy Chang
Assistant Research Director
Molecular Biology
CENTOCOR
244 Great Valley Parkway
Malvern, PA 19355

Dear Nancy:

We are pleased that our collaborative efforts are making progress. Your synthesis of HTLV-III env gene products using our HTLV-III DNA clone is encouraging. We are beginning to use these in our larger NCI vaccine research development effort.

However, it has come to our attention that some time ago your organization filed a patent on the synthesis and uses of the expressed products from our HTLV-III DNA clones which were designated for collaborative research. We found out that our names are not included on the patent, despite the fact that your use of the clone was indispensable to your part of the effort.

We assume that this was an oversight. We would like to ask that your patent be modified and that our names be added to your patent application. We feel that a formal recognition of our contribution is integral and that the inclusion of our names is only fair.

Sincerely yours,

Robert C. Gallo, M.D.

RCG/PF/bj

cc Dr. Chabner
Dr. DeVita
Dr. Fischinger
→Dr. Harmison
Dr. Sliski
Dr. Wall ✓

Documentary Exhibit 6
CHANG ET AL.
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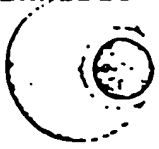
EXHIBIT C



244 GREAT VALLEY PARKWAY, MALVERN, PA 19355. (215) 296-4488
TELEX: 834823 CENTOCORMARN
FAX: 215-644-7558

August 5, 1985

David Brook, Esquire
Hamilton, Brook, Smith & Reynolds
Two Militia Drive
Lexington, Massachusetts 02173

Dear David:

I will respond to Dr. Gallo at the National Institutes of Health upon my return, August 20, 1985. In the meantime please discuss this matter with Nancy Chang regarding the facts surrounding this invention.

I believe Dr. Gallo mixes up inventorship with contribution. This issue is politically sensitive and I may wish to compromise. I will also discuss this with Dr. Lawless at Du Pont. Du Pont is licensed by the government.

Sincerely,

Hubert J.P. Schoemaker

Hubert J.P. Schoemaker, Ph.D.
President

HJPS:so'h
attachment
cc: Dr. Nancy Chang
Dr. Gregory Lawless

EXHIBIT D

CENTOCOR

244 GREAT VALLEY PARKWAY, MALVERN, PA 19355. (215) 296-4488
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September 16, 1985

Dr. Robert Gallo
National Institutes of Health
9000 Rockville Pike
Building 37
Room 6A09
Bethesda MD 20205

Dear Dr. Gallo:

I have in hand your letter of July 25, 1985 addressed to Dr. Nancy Chang regarding inventorship on the Centocor HTLV-III patents. There is no question that your collaboration was essential to the overall program and, as you know, we have on every occasion, made this fact clear.

In the case of the patent covering the assay development, our lawyers advised us that, under strict inventorship interpretation, your contribution should be referenced in the patent but that you should not appear as an inventor. These rules are quite contrary to the rules for authorship on scientific papers.

We wish to have the patent legally drawn. Anything to strengthen the patent is an advantage. If the lawyers feel your name should be added, we would be not only willing but anxious to have this accomplished.

I would be happy to discuss this matter with you or your representative or arrange to have our patent attorneys visit you in Washington. If you wish to talk to our attorney, please feel free to call David Brook of Hamilton, Brook, Smith & Reynolds directly at 617-861-6240. David does our patent work and his principal client is MIT. He is most qualified in the patent area.

Your work for the government and the community is outstanding. We have attempted to support you to our utmost in the past and will use our best efforts to support you in the future.

Yours very truly,

Hubert J.P. Schoemaker, Ph.D.
President

cc: D. Brook, Esq.
N. Chang, Ph.D.
H. Wall, Chairman

Documentary Exhibit 8
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Interference No. 103,659